HUMAN/ROBOTIC HEMATOPOIETIC STEM CELL THERAPY AND GENE THERAPY FOR EXPLORATION OF THE SOLAR SYSTEM

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SYNOPSIS

Long-duration space mission requires countermeasures against catastrophic disorders in astronauts, such as cancer, immunodeficiency, muscle and bone losses, hematological and cardiac abnormalities. Hematopoietic stem cell (HSC) therapy and gene therapy could provide ultimate countermeasures/cure to these illnesses. Methods of purification and culture of HSCs in 1 G are established in this laboratory. Adeno-associated virus (AAV)-mediated *ex vivo* gene therapy is yet to be completed, but underway. To enable these treatments by the crew-members in space, the following procedures need to be established and automated/robotized:

- **Engineering of the robotic HSC purification machine**
- **Automated device for growing hematopoietic stem cells (HSCs) in low/0 gravity from the frozen HSCs**
- **HSC delivery mechanisms:**
  - **a) Gastric resistant capsule form; automated packaging of HSCs in capsule**
  - **b) Intravenous injection: development of i.v. injection machine**
- **HSC therapy for muscle and bone losses:**
  - **a) Targeting of HSCs to the tissues**
  - **b) Study of the effect of HSC therapy on muscle and bone losses, using rodent hind-limb suspension model**
- **Establishment of ex vivo gene therapy protocols in space using a mouse model of β-thalassemia**
- **Human clinical trials for HSC therapy and gene therapy**

1. TECHNICAL DESCRIPTION

a. Abstract

Manned space missions to other planets require countermeasures against various disorders caused by low/zero gravity. Some, if not all, of these disorders may be amenable to hematopoietic stem cell therapy (HST) and gene therapy. The expertise and a collaborative network built by this laboratory to pursue the adeno-associated virus (AAV)-mediated gene therapy for β-thalassemia (Cooley's anemia), sickle cell disease, and AIDS, is useful to develop countermeasures against the space-caused, HSC-related disorders, *i.e.* anemia, immunodeficiency, and blood-born cancer, e.g., leukemia. In addition, the HSCs might prove to be useful to countermeasure muscle and bone losses. The long-term goal of this laboratory is to **automate/robotize** the HSC and gene therapy protocols so that astronauts would be able to treat themselves during the mission. Such procedures/devices would also benefit the people on earth as a self-care health system.

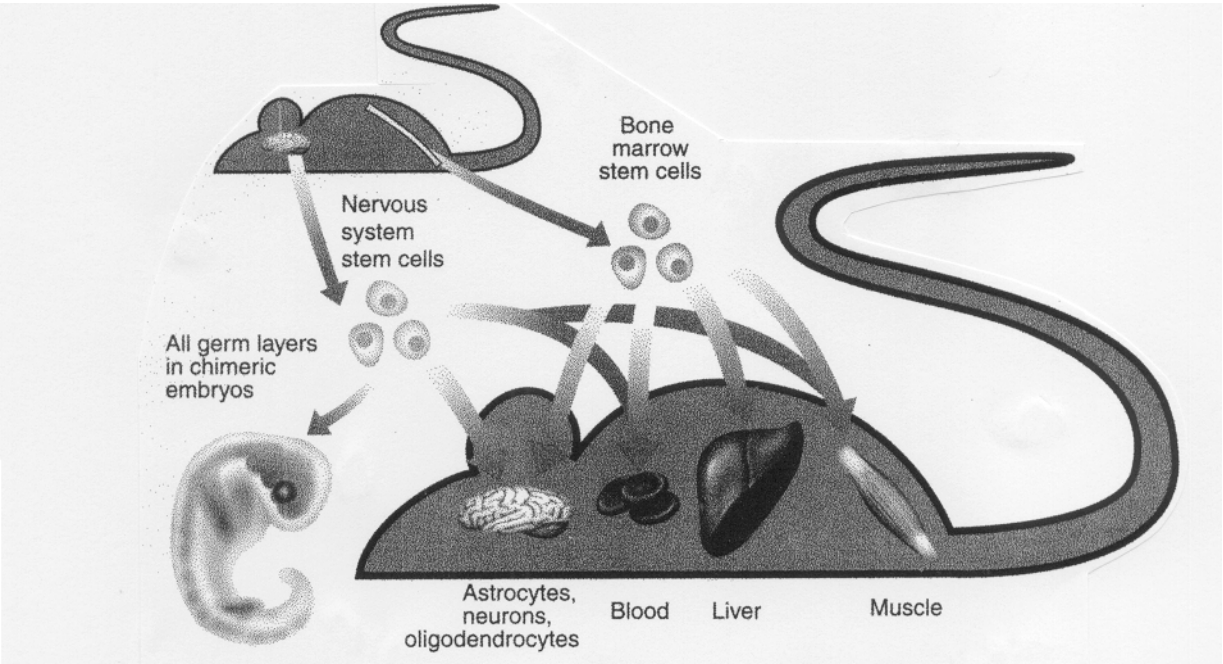
b. Concept Description

Long-term space exploration may result in catastrophic health problems among the astronauts, such as cancer, immunodeficiency, bone and muscle losses, hematological and cardiac abnormalities (1,2). Therefore, our long-term goal is to develop a robotic health management system that can be used by crewmembers without a physician or a health specialist during the mission. One avenue of this robotic medicine is a human/robotic HST and gene therapy systems, which could countermeasure the catastrophic disorders. Based on the ground-based experiments, we have been developing protocols for AAV- and HSC-mediated gene therapy of the hemoglobinopathies, β-thalassemia and sickle cell disease as well as AIDS (3-6). Therefore, it is our intension to modify the procedures to apply to the HSC-related disorders in space (6,19). Methods of purification and culture of HSCs in 1 G are established in this laboratory, and the AAV-mediated *ex vivo* gene therapy is yet to be completed, but underway.

Since the hemtopoietic stem cells give rise to all types of blood cells, the hematological disorders space anemia, immunodeficiency and bone marrow derived cancer, e.g. leukemia, may be amenable to HST and HSC-mediated gene therapy. For example, suppression of erythropoiesis by 0 G (7,21) and the astronauts' postflight anemia (8,9) may be treated by autologous HSC transplantation (HSCT). In order to countermeasure suppression of stem cell hematopoiesis, erythropoiesis and myelopoiesis by 0 G (7,20,21), bone marrow stromal cells could be genetically engineered to produce erythropoietin and other cytokines to promote hematopoiesis. If mean corpuscular hemoglobin concentration (MCHC) in red blood cells becomes low, the astronaut's HSCs could be transduced by our recombinant AAVs (rAAVs) harboring α- and β-globin cDNAs/gene and transplanted, resulting in more hemoglobin production in red cells. The immunodeficiency, which most likely results from suppressed myelopoiesis in 0 G, could be alleviated by autologous HSC transplantation. With regard to radiation-induced cancer, which is a serious threat to space missions, astronaut's HSCs might be engineered to become radiation resistant by transducing radiation resistant gene(s) by gene therapy. Alternatively or

concomitantly, we could transduce anti-cancer gene (tumor suppressor gene) to HSCs in order to protect from/cure the disease, as in the case of Neurofibromatosis (10,11). When cancer is treated by high-dose chemotherapy, the suppressed bone marrow could be repopulated by HST. The host HSCs could be engineered to become drug resistant by gene therapy as well (12). With regard to muscle, bone losses and cardiac abnormalities in 0 G, it is an intriguing possibility that the HSCT may countermeasure these disorders, too, since several laboratories recently reported that HSCs could differentiate to various tissues, such as blood, muscle, liver, lung, skin, GI tract, and neuronal cells, etc. (25,26; Fig. 1).

Fig. 1



Adapted from: Gretchen V, *Science* **290**:1674 (2000)

These findings open up a possibility that the HST may help to prevent or repair muscle, bone losses and cardiac abnormalities in space. Therefore, it is worthwhile to study this feasibility using a rodent hind-limb suspension model, and we are starting the collaboration with Dr. Jay R. Shapiro of Uniformed Services HealthSciences University/Walter Reed Army Medical Center. As such, numerous applications would emerge, once we establish procedures of HSC therapy and gene therapy in space. While the HSC transplantation is currently in use in clinical settings, albeit experimental stage, the ground-based procedures need to be modified to apply to the space situation step-by-step. So will be the gene therapy approach. In addition, currently, no robotic systems exist for these procedures. Therefore, major efforts are needed for the space biologists and engineers to collaborate for designing and executing the development in consultation with NASA and industries.

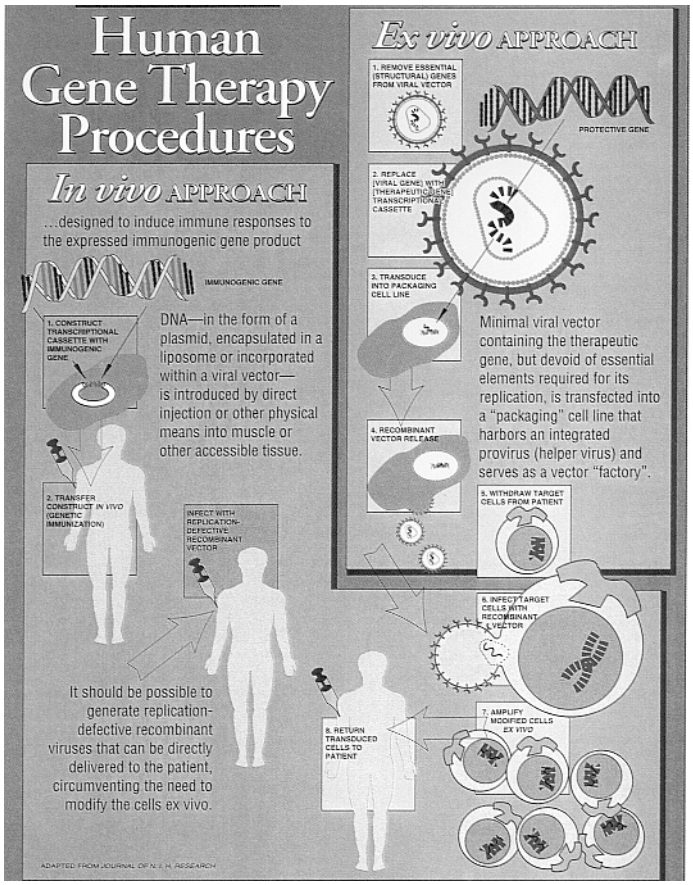
The relevant information on AAV-mediated gene therapy is as follows: The adeno-associated virus, serotype 2 (AAV2), is a human parvovirus that contains a linear single-stranded DNA of 4,675 nucleotides (nt), having broad host-range and tissue-specificity (13,14). Since there is no disease caused by this virus, in part because of the specific integration of wild-type AAV2 into the human chromosome $19q^{ter}$ (27,28), it is regarded as a **non-pathogen**. Our study on our own **recombinant** AAV also indicates specific integration into the mouse chromosome (30). Therefore, the virus promises to be a safer vector for gene therapy than other pathogenic viruses, such as, adenovirus, retrovirus and herpes virus, which are also being used as the gene transfer vectors. The non-pathogenicity of AAV2, compared with other vectors, is particularly important for space mission, because of the enclosed environment of the spaceship. As to the question of excision and replication of the chromosome-integrated recombinant AAV (rAAV), even if astronauts harbor the helper viruses, such as adenovirus or herpes virus, the recombinant AAV cannot replicate, since the rAAV genome lacks capsid gene regions. According to our study, we see no detectable amount of wild type AAV2 in our rAAV preparation (4,5). Even if contaminated wild type AAV2 existed, emergence of virulent strain by mutation is quite unlikely, considering billions of years of viral evolution: the AAV2 has remained non-pathogenic. If the worry persists, there could be an option to package recombinant AAV genome into "targetable" liposomes (29), avoiding production of the infectious AAV particles.

Exploiting these advantages of AAV2, starting in 1985, we have constructed rAAVs that harbor human globin genes as well as an anti-HIV-1 *gag* ribozyme (anti-*gag* Rz) for eventual use in gene therapies of the hemoglobinopathies and AIDS, respectively (3-5,18,30). More recently, our interest expanded to develop HST- and gene therapy-based countermeasures for space exploration (6,19,31). Many laboratories worldwide are now using the rAAV system for various gene therapy projects with favorable results (15-17). While we have obtained several key results on AIDS gene therapy, focusing on gene therapy of hemoglobinopathies, the following is a brief description of current status of this laboratory: To achieve *ex vivo* gene therapy for hemoglobinopathies, several procedures need to be delineated (Fig. 2, p. 5). These include, in the order of development: *(1)* construction of recombinant viral vectors that harbor globin cDNAs/genes; *(2)* characterization of the vectors in terms of infectivity, integration, and gene expression; *(3)* isolation and culture of hematopoietic stem cells (HSCs); *(4)* transduction of the HSCs with viral vectors and subsequent expression of globin polypeptides from the constructs *in vitro* (cell culture); *(5)* transplantation of the virus-transduced HSCs to animal models of hemoglobinopathies, resulting in; *(6)* efficient gene expression *in vivo* and correction of the hemoglobinopathies by gene therapy; *(7)* clinical trials with human patients. Among these steps we have successfully established procedures up to the step 4. In addition, the HSC transplantation procedure, an important part of the step *(5)* above, has been recently established (18,19). The detailed descriptions on these steps are omitted from this application because of page limitations, but are available upon request. Putting all the techniques together, we are currently trying to cure mouse model of human β-thalassemia (18,19). Once these mice are cured by gene therapy, we will then move to possible clinical trials for human patients.

Since globin-AAV vectors are already constructed in this laboratory, and since the procedures for purification, culture and transplantation of HSCs under 1 G condition are available, the first step

in the space program will be to establish optimal conditions for HSC growth and HSC transplantation (HST) in space, because these HSCs become primary cells to transduce with rAAVs. We consider this step to be particularly important, since the HSC growth and

Fig. 2



Adapted from: *J NIH Research*

hematopoiesis in general are reported to be inhibited in 0 G environment (7,20,21). If we are to develop HST and gene therapy in space using the HSCs, it is imperative to develop methods to overcome the growth inhibition. Our interest has been to use the NASA Rotating Wall Vessel (RWV) and Rotating Wall **Perfused** Vessel (RW**P**V) to culture HSCs, because the RWV, aside from the low gravity simulating effect, may have a possible beneficial effect on the growth of HSCs. Our experience on the suspension culture of HSCs indicates a beneficial role of gently mixing the culture at least once a day. We believe that such a condition may reflect *in vivo* status of HSCs; namely, while these cells are carefully protected by the bone sheath, they are in a constantly moving environment since the body moves. This mobile environment may provide the active cell-to-cell contact or cell-to-growth factor interaction, which the RWV/RWPV appears to provide.

To establish the optimal conditions for growth of HSCs, we have been collaborating with the

NASA-NIH Center for Three-Dimensional Tissue Culture, which possesses and operates the RWV culture (contact persons: Leonid B. Margolis, Ph.D. and Wendy Fitzgerald, B.S.). We have performed some preliminary studies to establish the RWV culture for mHSCs (6,19,31), employing cell count study and flow cytometry with stem cell-specific antibodies. With 30 rpm RWV and the prescribed medium, encouraging initial results are obtained, indicating some beneficial effect of the RWV, and now more extensive analysis of the cells is needed. The expansion of mHSCs in the RWV culture will be optimized by adjusting rotation rate of RWV, frequency of medium change, and addition of other cell populations and growth factors/cytokines etc. In addition, systematic analysis of cells retrieved from the RWV cultures will be conducted using flow cytometer and clonogenic assay. Stem cell specific and lineage-specific antibodies will be used to characterize the expansion and differentiation. Clonogenic assay in the methyl cellulose culture is routinely done in this laboratory. Other laboratories are also reporting the use of RWV culture system for mouse HSC (20) and erythroleukemia cell line (21), with apparent inhibitory effect of the RWV on the cell growth and differentiation. However, as the isolation methods and culture methods of HSCs are different in each laboratory, it is imperative to delineate the best conditions for our own preparations. In addition, since multiple steps are involved in gene therapy protocols, it is necessary to develop conditions that suit our needs, so that one controlled flow system is available to accomplish gene therapy. Thus, with solid funding mechanisms, the optimization of HSC growth in RWV will be continued in collaboration with the NASA-NIH Center to the extent that the process would be robotized. We have not studied the Rotating Wall Perfused Vessel (RWPV) system yet and may be worth studying, also. In addition, the NASA Hypergravity Facility for Cell Culture (HyFACC) system, developed by NASA Ames Research Center, is of interest for us to study. The centrifuge culture system would provide 1 G condition to the HSCs in a static culture system during 0/low G spaceflight environment, closely mimicking the earth condition we routinely utilize. In addition, the equipment is useful to study the effect of hypergravity on HSCs, simulating the condition of shuttle launching the astronauts experience. Through email correspondences with staff members of NASA Ames Research Center (Gary Jahns, Jim Connolly, Tianna Shaw), an arrangement has been made to initiate these studies upon the grant funding.

Other cell/tissue culture equipment NASA developed would be incorporated to delineate the optimum condition of growing and expanding the HSCs. Among the issues are procedures of cryopreservation and reculture of HSCs, if we were to use frozen HSCs. This project will have practical applications, such as cryopreserving astronauts' HSCs before the flight and growing/expanding them in the International Space Station (ISS) or spaceship, as the need for HST and gene therapy arises. This part of the study relates to automation of HST and gene therapy. When the frozen HSCs would not grow or died, it may become necessary to prepare HSCs from astronaut's peripheral blood. Therefore, in this RFI, it is proposed to create a HSC purification machine, which currently does not exist at all on the earth; all are done manually. The machine need to be robotized so that crew members can operate by simply punching the buttons. Furthermore, the robot needs to be modified to function in 0/μ G. This is certainly a challenging task, but could be done by concerted efforts of biologists and engineers.

Since we have a colony of β-thal mice, quality of the RWV or HyFACC cultured HSCs can be assessed by their ability to cure β-thalassemic mice by HSC transplantation (HSCT). With the optimum condition established for HSC expansion, the next phase would involve curing the β-thalassemic mice by rAAV-mediated gene therapy. Conducting the HST and gene therapy experiments with the β-thal mice in the International Space Station will help determine many parameters. Of course, the information obtained for curing mouse model of β-thalassemia will serve as the base for developing protocols of eventual **human** gene therapy in space. Successful growth and expansion of mouse HSCs in the μ G environment will promote the similar experiments with human peripheral blood hematopoietic stem cells (PBHSCs), which we routinely isolate.

In conclusion, the specific aims of the present project are as follows:

1) Toward HST and rAAV-gene therapy in space, conditions of HSC growth and expansion will be established using the NASA Rotating Wall Vessel (RWV) culture system and the Hypergravity Facility for Cell Culture (HyFaCC) system.

2) The quality of expanded HSCs will be assessed by their ability to cure β-thalassemic mice by HSC transplantation.

3) Conditions of cryopreservation and reculture of HSCs will be established. These cell should serve as the source of the HST and gene therapy.

4) Effects of HSC therapy on muscle, bone losses and cardiac abnormalities need to be studied. The rodent hind-limb suspension model should be useful for the study.

5) The HST and AAV-gene therapy procedures need to be robotized.

2. MANAGEMNET APPROACH

Principle Investigator: Seigo Ohi, Ph.D.

Co-Investigators:

Leonid B. Margolis, Ph.D., Section Head, NASA-NIH Center for Three-Dimensional Tissue Culture, Bethesda, MD

Wendy Fitzgerald, B.S., Senior Scientist, NASA-NIH Center for Three-Dimensional Tissue Culture, Bethesda, MD

Oswaldo Castro, M.D., Professor and Director, Center for Sickle Cell Disease, Howard University College of Medicine and Hospital, Washington, DC

Bak C. Kim, B.S., Research Associate, Center for Sickle Cell Disease, Howard University College of Medicine and Hospital, Washington, DC

Patricia A. Dinndorf, M.D., Professor and Staff Member, Children's National Medical Center and George Washington University School of Medicine, Washington,

DC

The Principal Investigator for this project is Seigo Ohi, Ph.D. and is responsible for advancing the present proposal. Mr. Bak C. Kim, Research Associate has been assisting Dr. Ohi for several years in the past and will continue to participate in this project. Ohi and Kim have been collaborating with Leonid Margolis, Ph.D. and Wendy Fitzgerald, B.S. in the NASA-NIH Center for Three-Dimensional Tissue Culture since February 1999 to culture mouse hematopoietic stem cells (mHSCs) in the RWV system. The collaboration has been carried out as follows: Ohi and Kim sacrifice mice and prepare mHSCs and transport it to the NIH at 0^{o}C (ice bucket). All the media, cytokines and fluorescent antibodies have been provided by the P.I. Then, Fitzgerald, in consultation with Margolis, takes over and starts culturing HSCs in the RWV system. At the start of the culture, she divides cells into two halves and one is cultured in a static culture and the other in RWV culture. At the start of culture and once a week thereafter, she takes aliquot of the culture and measures total cell numbers and also analyze expansion of the HSCs by flow cytometer they have. The results are reported to Ohi by email and fax. The email and fax have been extensively used during the course of study and serve as excellent communication media. This type of collaboration will continue in the present proposal.

To analyze the characteristics of HSCs, the P.I. has established collaboration with two hematologists, Oswaldo Castro, M.D. and Patricia Dinndorf, M.D. Castro is an expert of sickle cell disease, whereas Dinndorf is an expert of bone marrow and stem cell transplantation and therefore, will serve as Consultants in the mHSC study. Their enthusiastic willingness to participate in this project is seen in their letters of collaboration. The P.I. and Castro are staff members in Sickle Cell Center at Howard U., therefore, closely communicate each other. The P.I. contacts Dinndorf by email and telephone, as needed. For example, she helped provide the P.I. a current protocol of HSC transplantation. When the project progresses to use human subjects, both Castro and Dinndorf will become important active participants to this gene therapy project in the future. Close proximity of participants is an important asset to this project, since a meeting can be called up as the important issues arise.

In this project, the RWV cultured HSCs will be transplanted to β-thalassemic mice for curative study. To carry out this type of experiment, the HSCs will be harvested by Fitzgerald and brought back to the P.I.'s laboratory at Howard. Then, Ohi and Kim will carry out the HSC transplantation. Ohi has been working on the HSC transplantation in mice since 1994 and mastered the technique. To analyze the hemoglobin species of the mice, Ohi removes blood samples from mice via retroorbital sinuses and hand to Kim. Kim analyzes the blood samples by cystamine-cellulose electrophoresis. Analysis of hematological parameters is carried out in the Howard University Hospital, Hematology Laboratory. In addition, Ohi and Kim are breeding and maintaining β-thalassemic mice at Howard U. Veterinary Service Facility. Graduate students and rotating M.D./Ph.D. students are expected to participate in this project.

The automation/robotization of HSCT and gene therapy is a no easy task, but could be done by concerted efforts of biologists and engineers. The robot needs to be modified to function in 0/μ G. When the frozen HSCs would not grow or died, it may become necessary to prepare HSCs

from astronaut's peripheral blood. Therefore, in this RFI it is proposed to create a HSC purification machine, which currently does not exist at all on the earth. The machine needs to be robotized so that crewmembers can operate by simply punching the buttons. The current team is strong in biological research, but lack engineering skills. One avenue of solving this problem is to collaborate with Johns Hopkins University Applied Physics Laboratory and/or industries, such as SHOT (Space Hardware Optimization Technology, Inc.). The PI is discussing the collaboration with Dr. Paul W. Todd, Program Manager Bioprocessing, SHOT.

Biographical Sketch of the PI:

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EDUCATION/TRAINING:

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Princeton University, Princeton, NJ - Ph.D., Molec. Biol., 1973. Supervisor: N. Sueoka, Ph.D., Guggenheim Professor of Molecular Biology

Carnegie Inst. of Washington, Baltimore, MD - Postdoc., Molec.Gentics, 1973-75. Sponsor: I.B. Dawid, Ph.D., Staff Member and Professor, Johns Hopkins U.

PROFESSIONAL EXPERIENCE:

1975-78 Research Associate, University of Pittsburgh School of Medicine, Dept. of Anatomy and Cell Biology. Sponsor: Irving Lieberman, Ph.D., Professor.

1978-83 Research Associate, Johns Hopkins University School of Hygiene and Public Health, Dept. of Biochemistry. Sponsors: Lawrence Grossman, Ph.D., Professor and Chairman and P.C. Huang, Ph.D., Professor.

1983-86 Fogarty Visiting Associate, NIAID-NIH, Laboratory of Biology of Viruses. Sponsor: James A. Rose, M.D., Section Head.

1986-91 Assistant Professor and Staff Member, Meharry Medical College School of Medicine and Grad. Sch. of Arts and Sciences, Dept. of Biochemistry, NIH Comprehensive Sickle Cell Center.

1991-93 Assistant Professor and Molecular Geneticist, Howard University College of Medicine and Grad. Sch. of Arts & Sci., Dept. of Biochemistry and Molecular Biology, Center for Sickle Cell Disease.

1993- Associate Professor and Molecular Geneticist, ibid. and Dept. of Genetics & Human Genetics

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